0022-1767/84/1336-2972802.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 133, No. 6, December 1984
Printed in U.S.A.

DEFECTIVE NATURAL KILLER CYTOTOXICITY AND POLYMORPHONUCLEAR LEUKOCYTE ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY IN PATIENTS WITH LFA-1/OKM-1 DEFICIENCY[1]

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Four children with an immunodeficiency involving the absence of leukocyte membrane glycoproteins reacting with anti-LFA-1 and OKM-1 monoclonal antibodies were unable to mediate adherence-dependent leukocyte functions. Even with normal Fc receptor function, their PMN-ADCC and MC-NKC were markedly deficient. Single cell analysis demonstrated deficient antibody-mediated PMN-target cell adherence. Monoclonal antibodies against LFA-1 and OKM-1 reproduced this immunodeficiency in leukocytes from normal adults. LFA-1/OKM-1 mediates a PMN-target cell adhesive step.

Cellular cytotoxicity is a leukocyte-mediated extracellular killing mechanism. There are several types of cytotoxicity including H-2 or HLA-restricted T cell cytotoxicity, natural killer cytotoxicity (NKC),[3] and antibody-dependent cellular cytotoxicity (ADCC). These are mediated by specifically sensitized T cells, natural killer (NK) cells, and a variety of ADCC-competent cells, respectively. Cytotoxicity occurs against a plethora of targets including nucleated and non-nucleated eucaryotic cells, virus-infected cells, fungi, bacteria, and other parasites. The mechanism of cytotoxicity has been shown to involve several steps. These include target recognition, binding, programming to kill, effector cell-independent target cell lysis, and effector cell recycling (1–8). Several important leukocyte membrane structures have been elucidated that are essential for cytotoxicity. For ADCC the IgG Fc receptor is critical (8). For T cell cytotoxicity, a membrane glycoprotein LFA-1 as well as the antigen receptor on the effector cell is essential, and HLA antigens on the target cell are important (9–14). There is increasing evidence that sugar receptors and LFA-1 are involved in NKC (6, 14–16).

We studied a series of patients with a well-characterized leukocyte immunodeficiency syndrome. This syndrome involves the absence of a family of leukocyte membrane glycoproteins LFA-1 and OKM-1 which have been shown to be critical for several adhesive-mediated functions (17–19). A third member of the family, P150,95, which has not yet been functionally characterized, is also missing. Members of this glycoprotein family are found on human T lymphocytes, polymorphonuclear leukocytes (PMN), macrophages, and NK cells (20, 21). We now demonstrate that even in the presence of functional Fc receptors, leukocytes from patients that lack LFA-1/OKM-1 fail to generate PMN-mediated ADCC and lymphocyte-mediated NKC. Functional analysis has revealed this failure to be due to a defect in effector cell-target cell binding in the case of PMN-ADCC. The use of specific monoclonal antibodies (MAb) to OKM-1 and LFA-1 has confirmed the critical role of these glycoproteins in PMN-ADCC and lymphocyte-NKC of cells from normal individuals. This is the first demonstration in humans of a link between a leukocyte membrane molecular defect and functional leukocyte cytotoxic defects.

MATERIALS AND METHODS

Patients. Four unrelated patients, a 5-yr-old female (number one), an 11-mo-old female (number two), a 15-mo-old female (number three), and a 17-yr-old male (number four) were studied. Each manifested recurrent soft-tissue infection, progressive peridontitis, and persistent granulocytosis, and most had delayed umbilical cord separation at birth. Severe defects in leukocyte mobilization *in vivo* (Rebuck skin window), leukocyte motility *in vitro* (Boyden chamber assay) were identified. Further evaluation demonstrated profound abnormalities in adherence-dependent PMN and monocyte functions including cell attachment and spreading, aggregation, orientation, and phagocytic ingestion of particles opsonized with C3-derived ligands. SDS-PAGE of PMN lysates of each patient demonstrated a severe deficiency or total absence of an identical high m.w. series of glycoproteins. As shown with a NaB^3H_4 galactose oxidase labeling technique, PMN of each patient lacked a major surface glycoprotein or glycoprotein complex. Subsequent studies employing specific MAb demonstrated a severe deficiency or total absence of LFA-1 and OKM-1 (17, 18).

Effector cells. Venous blood samples were sedimented in 0.1 ml of 6% dextran and 0.87% NaCl/10 ml of blood. Leukocyte-rich plasma was centrifuged at 300 × G for 5 min, and leukocytes were resuspended in Dulbecco's phosphate-buffered saline (DPBS) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), pH 7.4, containing 0.2% dextrose. Cells were layered over a solution of Ficoll-Hypaque containing 10 parts of 33.9% Hypaque (Winthrop Laboratories, New York, NY) and 24 parts of 9% Ficoll (Sigma Chemical Co.,

Received for publication May 24, 1984.
Accepted for publication July 26, 1984.
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[1] This work was supported by National Institutes of Health Infant Nutrition Grants HD 13021, AI-19031-02, Ko4 AI 00501-01, and CA 31798.
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[3] Abbreviations used in this paper: ADCC, antibody-dependent cellular cytotoxicity; NKC, natural killer cytotoxicity; DPBS, Dulbecco's phosphate-buffered saline; PMN, polymorphonuclear leukocytes; MC, mononuclear cell; MAb, monoclonal antibody.

St. Louis, MO), centrifuged at 800 × G for 30 min. Mononuclear cells (MC) in the interface layer and PMN in the cell button were washed twice in DPBS. Cell viability as determined by trypan dye exclusion was greater than 95%. Final PMN suspensions contained greater than 97% PMN and minimal platelet contamination. The erythrocyte to PMN ratio was less than 3:1. Cell preparations were adjusted to a concentration of 10^7 MC or PMN/ml DPBS. To lyse the red blood cells, the sediment containing the PMN was resuspended in 2.5 ml of distilled water and was pipetted for 45 sec, and then 2.5 ml of twice-normal saline was added, and again the suspension was pipetted for 45 sec. Cells were purified and maintained at 4°C before their utilization in all assays. The cell suspensions were washed three times in Hanks' balanced salt solution (HBSS; Gibco) and suspended in minimal essential medium (MEM). The separated MC obtained at the interface were similarly washed three times in HBSS and resuspended in MEM.

Target cells. As previously described in detail (22), target cells were Chang liver cells infected with herpes simplex virus (HSV) type 1 (multiplicity of infection of approximately 1) the day before use and labeled with radioactive sodium chromate (New England Nuclear, Boston, MA) the day of use.

K562 cells were kindly provided by Dr. Eva Lotzova (M.D. Anderson Hospital and Tumor Institute, Houston, TX) and were handled as above, except for viral infection.

Sera. Immune serum consisted of a pool of sera prepared from four individuals with past HSV infections. The presence of HSV antibodies was assayed by means of neutralization and lymphocyte-mediated ADCC. The neutralizing antibody titer of the pool was 1/32, and the lymphocyte-ADCC titer was 10^{-5}. Nonimmune sera consisted of pooled sera from four individuals with no history of HSV infection and no detectable HSV antibodies, as determined by neutralization or ADCC. All sera were heat-inactivated a 56°C for 30 min and were stored at −40°C before use.

Microcytotoxicity assay. The anti-HSV microcytotoxicity assay was performed in triplicate in rigid-polystyrene U-bottomed-well microtiter plates (Cooke, Alexandria, VA) (22). Effector cells with concentrations adjusted to provide effector to target cell ratios from 30:1 (1.5×10^5/well) to 100:1 (5×10^5/well), target cells (5×10^3 cells), and serum were added to the wells. The final serum concentration was 1/20 for PMN and 1/100 for MC. These concentrations previously were determined to promote maximal antibody-dependent cellular cytotoxicity for each respective cell population. The covered plates were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 18 hr. NKC to K562 was performed in an identical manner except for the lack of antiviral serum in the assay, and the use of a 4-hr incubation period.

To determine the amount of ^{51}Cr released from the target cells, 100 μl was aspirated from the top of each well without disturbing the cell button. To each well, 100 μl of 1 M NaOH was added, and the total volume was aspirated into a separate container. Each sample was counted in a Beckman 4000 gamma counter for 1 min. ^{51}Cr release was calculated as

$$\text{percent } ^{51}\text{Cr release} = \frac{2A}{A + B} \times 100$$

in which A = counts per minute in the top 100 μl, and B = counts per minute in the bottom 100 μl to which NaOH was added. ADCC was calculated as

$$\text{percent ADCC} = \frac{\begin{array}{l}(\text{percent } ^{51}\text{Cr release of target cells} \\ \quad + \text{effector cells} + \text{immune sera}) \\ \quad - (\text{percent } ^{51}\text{Cr release of target cells} \\ \quad + \text{effector cells} + \text{nonimmune sera})\end{array}}{\begin{array}{l}100 - (\text{percent } ^{51}\text{Cr release of target cells} \\ \quad + \text{effector cells} + \text{nonimmune sera})\end{array}} \times 100$$

NKC was calculated as

$$\text{percent NKC} = \frac{\begin{array}{l}(\text{percent } ^{51}\text{Cr release of target cells} \\ \quad + \text{effector cells} + \text{nonimmune sera}) \\ \quad - (\text{percent } ^{51}\text{Cr release of target cells} \\ \quad + \text{nonimmune sera})\end{array}}{\begin{array}{l}100 - (\text{percent } ^{51}\text{Cr release of} \\ \text{target cells} + \text{nonimmune sera})\end{array}} \times 100$$

Each assay was performed in triplicate with SD of less than 10%. The spontaneous 18-hr ^{51}Cr release of target cells in the presence of immune or nonimmue serum alone was 30.4 ± 2.9%.

Single cell agarose assay. The single cell agarose assay was performed with minor modifications as reported by Silva *et al.* (23). This assay in our laboratory utilized unlabeled HSV-infected Chang liver target cells (2×10^5 cells/100 μl) mixed with leukocyte effector cells (1:1 effector to target cell ratio) in a final volume of 200 μl in 12 × 75-mm glass tubes. After 5 min incubation at 37°C and 5 min centrifugation (200 × G), the supernatant was removed and the cell sediment was suspended in 50 μl of MEM, resuspended ten times with an Ependorf micropipette, and was mixed in an additional 100 μl of 1.0% agarose (Type I; Sigma) in RPMI (Gibco) at 40°C. This entire mixture was spread on a bactoagar (Difco, Detroit, MI) precoated microscope slide and incubated horizontally in MEM at room temperature for 18 hr. Slides were then stained with trypan blue and were fixed in 0.5% formalin. Chang liver cells were easily distinguished from leukocyte effector cells by their larger size. Slides were read and coded, and 200 leukocytes were counted to determine the number of leukocytes binding to target cells.

Polyclonal antibodies and Mab. The OKM-1 MAb were obtained from Ortho Pharmaceutical (Raritan, NJ). The TS1/22 MAb to LFA-1 and the TS1/18 MAb to the common β-subunit of LFA-1 and OKM-1 were produced as previously described (13). $F(ab')_2$ fragments of MAb to LFA-1 were prepared by using pepsin digestion (24). A $F(ab')_2$ fragment of polyclonal rabbit IgG directed against the human C3b receptor (anti-CR1) was a generous gift of Dr. Douglas Fearon, Brigham and Women's Hospital, Boston, MA (25).

Fluorescence-activated cell sorter (FACS) analysis. Indirect immunofluorescence studies of intact PMN or MC were performed using MAb and fluorescein-conjugated anti-mouse IgG $F(ab')_2$ or goat anti-rabbit IgG $F(ab')_2$. Surface-stained cells were fixed in 1% paraformaldehyde and were processed in a Coulter Epics V flow cytometer (Hialeah, FL). FACS analysis was utilized to ensure specificity of binding and to determine saturable binding concentration with all MAb before their use in blocking experiments (17). At the concentration utilized, the anti-CR-1 antibody saturated PMN CR1 sites, as determined by FACS analysis (17).

Incubation conditions for blocking experiments. PMN or MC were incubated with MAb immediately after purification. Cell suspensions (2.5×10^7 PMN or MC in 1.0 ml DPBS) were incubated with or without MAb or their $F(ab')_2$ fragments for 30 min at 21°C. Final concentrations of polyclonal or MAb employed were OKM-1, 5 μg/ml; LFA-1-α $F(ab')_2$, 10 μg/ml; $F(ab')_2$ fragment of the β-subunit, 10 μg/ml; OKM-1 and LFA-1-α 10 μg/ml of each; and polyclonal rabbit anti-CR1 $F(ab')_2$, 5 μg/ml. For most experiments, cell suspensions were washed in DPBS before their use in cytotoxicity assays. In several experiments, additional MAb or their $F(ab')_2$ fragments were added to reaction mixtures of cytotoxicity assays to achieve a final concentration similar to that used during the initial incubation.

Statistical Analysis. Data are expressed as the mean ± SEM. Differences between patients and simultaneously tested controls were compared by using Student's two-tailed paired *t*-test.

RESULTS

PMN-ADCC. The ability of leukocytes from four patients with LFA-1/OKM-1 deficiency to mediate antiviral cellular cytotoxicity is displayed in Table I. At a variety of effector to target cell ratios all patients' PMN were markedly deficient in their ability to mediate ADCC. The mean ADCC values of patients' PMN were significantly lower than that of controls at effector to target cell ratios of 100:1 (patients, 4.4 ± 1.2; controls, 29.5 ± 8.1, $p < 0.025$), 60:1 (patients, 3.4 ± 1.2; controls, 24.8 ± 7.9, $p < 0.05$), and 30:1 (patients, 3.5 ± 1.0; controls, 18.3 ± 6.3, $p < 0.025$). Thus, PMN from these patients had a profound ADCC defect.

To determine whether defective PMN-ADCC by the patients' cells could be overcome by increasing the amount of antibody bound to target cells, PMN-ADCC assays were performed utilizing higher concentrations of antibody (Fig. 1). Whereas previous assays were performed by using final serum concentrations of 1/20 (Table I), experiments with two patients and paired controls were performed utilizing serum concentrations as high as 1/4. The higher serum concentrations increased PMN-ADCC levels of cells from normal individuals by nearly 100%, indicating a more dense IgG sensitization of target cells.

TABLE I

Cellular cytotoxicity of patients with leukocyte LFA-1/OKM-1 deficiency to HSV-infected cells

Patient/Assay	E:T[a]	PMN-ADCC[b]		MC-ADCC[c]		MC-NKC[d]	
		Patient	Control	Patient	Control	Patient	Control
1/A[e]	100	6.9	65.5	—	—	—	—
	60	7.2	60.9	6.9	—	1.2	—
	30	5.1	48.5	6.0	54.5	8.6	71.0
1/B[f]	100	6.6	34.6	—	—	—	—
	60	5.2	28.4	—	—	—	—
	30	1.2	17.4	—	—	—	—
2/A[g]	100	4.0	13.2	33.0	72.5	7.2	59.2
	60	0.3	15.9	23.0	47.5	6.1	53.1
	30	5.4	12.8	13.1	35.7	4.6	31.1
2/B[h]	100	0	19.2	37.4	89.4	11.1	79.4
	60	2.4	13.3	21.5	71.7	6.4	67.0
	30	6.0	8.7	24.3	69.9	0	40.4
3/A[i]	100	2.0	31.5	41.4	47.5	9.7	71.0
	60	5.0	23.2	32.6	43.3	11.5	57.5
	30	3.2	17.9	25.4	27.9	11.5	41.7
4/A[j]	100	7.1	12.3	82.7	43.2	33.9	22.7
	60	0	6.8	70.4	43.7	40.1	17.3
	30	0	4.6	70.4	38.1	25.5	13.8

[a] Effector to target cell ratio in the cytotoxicity assay.
[b] PMN-ADCC in an 18-hr ^{51}Cr-release assay.
[c] MC-ADCC in an 18-hr ^{51}Cr-release assay.
[d] MC-NKC in an 18-hr ^{51}Cr-release assay.
[e] Patient 1, assay A, the control was an unrelated adult.
[f] Patient 1, assay B, the control was an unrelated adult.
[g] Patient 2, assay A, the control was her brother.
[h] Patient 2, assay B, the control was an unrelated adult.
[i] Patient 3, assay A, the control was an unrelated adult.
[j] Patient 4, assay A, the control was an unrelated adult.

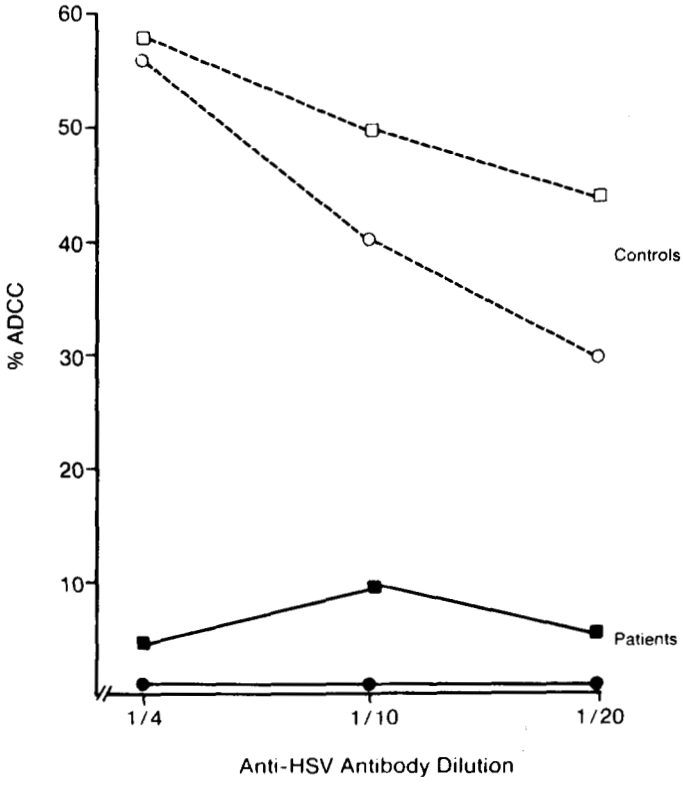

Figure 1. The effect of increasing concentrations of antibody-containing serum on PMN-ADCC of cells from patients with LFA-1/OKM-1 deficiency. The assay utilized an 18-hr incubation period and a 100:1 effector to target cell ratio. ●—● and ■—■, PMN from patients with LFA-1/OKM-1 deficiency; O- - -O and □----□, PMN from unrelated adult controls.

The PMN of patients with LFA-1/OKM-1 deficiency remained unable to mediate ADCC (Fig. 1). Thus, lack of PMN-ADCC of patients' cells occurred over a broad range of target cell antibody sensitization.

To further analyze possible mechanisms responsible for this defect, the ability of the PMN from LFA-1/OKM-1-deficient patients to adhere to HSV-infected target cells was measured in a single cell agarose assay (Table II). Whereas anti-HSV IgG increased target cell binding of PMN from normal individuals (from 1.5 ± 1.2% to 4.0 ± 2.0%, $p < 0.01$), no increase in adherence was induced by antibody in the presence of PMN from patients with LFA-1/OKM-1 deficiency (1.2 ± 0.2% to 1.0 ± 0.2% in the presence of antibody). The difference in the percentage of cell adherence in the absence and presence of antibody (delta adherence) between patients with LFA-1/OKM-1 deficiency (0.08 ± 0.1) and controls (3.2 ± 0.9) was significant ($p = 0.01$). Thus, the PMN-ADCC defect was due, at least in part, to an inability of PMN to adhere to target cells in the presence of antibody.

To be certain that the 18-hr single cell assay accurately reflected the percentage of PMN binding to target cells, assays with cells from normal adults were performed utilizing a 1- to 4-hr incubation period. In six assays the percentage of PMN adhering to target cells in the presence of antibody was 3.4 ± 0.7%, and the percentage of PMN adhering to target cells in the absence of antibody was 1.3 ± 0.3%. The viability of the PMN by trypan dye exclusion in the 18-hr assay was 97.3 ± 0.1%, and the viability of the PMN adherent to target cells was 90.3 ± 1.0%. Thus, the small number of adherent cells was due not to lysis of PMN during the 18-hr incubation, but reflected the true percentage of adherent PMN. This is to be expected because a high effector to target cell ratio is required to mediate PMN-ADCC in a ^{51}Cr-release assay (Table I), even though kill is mediated by a single PMN lethal hit.

MC-ADCC. As shown in Table I, patients were able to mediate MC-ADCC to HSV-infected cells in a nearly normal fashion. Although the mean MC-ADCC of patients' MC at effector to target cell ratios of 100:1 (patients, 48.6 ± 11.5; control, 63.2 ± 10.8), 60:1 (patients, 30.9 ± 10.7; controls, 51.6 ± 6.7), and 30:1 (patients, 27.8 ± 11.2;

TABLE II

Cell binding in single cell agarose assay of patients with leukocyte LFA-1/OKM-1 deficiency

Patient/Assay	PMN[a]				MC[b]			
	Patient		Control		Patient		Control	
	Antibody[c]	No antibody[d]	Antibody[c]	No antibody[d]	Antibody[c]	No antibody[d]	Antibody[c]	No antibody[d]
1/A[e]	0.5	1.5	6.0	0.5	2.0	1.5	5.5	1.5
1/B[f]	0.5	0.5	4.5	2.5	4.0	1.0	5.0	1.0
2/A[g]	1.0	1.0	2.0	1.0	3.5	1.5	3.0	2.0
2/B[h]	0.5	1.0	2.0	0.5	5.0	1.5	5.5	1.0
3/A[i]	2.0	1.5	6.5	3.5	3.0	2.0	7.5	3.5
4/A[j]	1.5	1.5	6.0	1.5	3.0	1.5	12.5	2.5

[a] The results are the percentage of PMN binding target cells in 18-hr single cell agarose assay.
[b] The results are the percentage of MC binding target cells in 18-hr single cell agarose assay.
[c] The percentage of cells bound in the presence of 1/100 dilution of anti-HSV antibody-containing serum.
[d] The percentage of cells bound in the presence of 1/100 dilution of serum not containing anti-HSV antibody.
[e] Patient 1, assay A, the control was an unrelated adult.
[f] Patient 1, assay B, the control was an unrelated adult.
[g] Patient 2, assay A, the control was an unrelated adult.
[h] Patient 2, assay B, the control was an unrelated adult.
[i] Patient 3, assay A, the control was an unrelated adult.
[j] Patient 4, assay A, the control was an unrelated adult.

controls, 45.2 ± 7.5) were lower than that of controls, these values were not statistically significantly different.

As would be expected, concomitant with low but essentially normal levels of MC-ADCC, in the single cell agarose assay (Table II), antibody induced the patients' cells to form significantly more adherent pairs (3.4 ± 0.4, as compared to 1.5 ± 0.1 in the absence of antibody, $p = 0.01$. If cells from adults were used, similar results were seen (6.5 ± 1.3 in the presence of antibody compared with 1.9 ± 0.4 in the absence of antibody, $p = 0.02$). As with ADCC values, percent adherence tended to be higher if cells from adults were used, but the difference in antibody-mediated adherence (delta adherence) was not statistically significant between the patients and controls.

Thus, unlike PMN, MC from patients with LFA-1/OKM-1 deficiency were able to utilize specific antibody and other cell surface components to adhere to target cells and mediate ADCC.

MC-NKC. MC-NKC of the patients with LFA-1/OKM-1 deficiency was markedly lower than that of control MC in the first three patients (one, two, and three). Patient four had NKC values within the adult range at all effector to target cell ratios tested (Table I). At an effector to target cell ratio of 30:1, the mean MC-NKC of patients' cells (10.0 ± 4.3) was significantly ($p < 0.05$) lower than that of controls (39.6 ± 9.3).

In the single cell assay there was no significant difference in percent MC adhering to HSV-infected target cells in the absence of antibody (patients, 1.5 ± 0.1, and controls, 1.9 ± 0.4; Table II).

Interferon enhances lysis but not binding in NKC (6, 23). Therefore, interferon was utilized to determine whether it could increase lysis in cells with a binding defect. Interferon (10^3 U of IFLrA, recombinant DNA human α-interferon, kindly provided by Hoffman-La-Roche, Nutley, NJ) added to MC (effector to target cell ratio of 60:1) 2 hr before use in the cytotoxicity assay, and present throughout, increased MC-NKC of three patients (one, three, and four) from 12.3 ± 10.2 to 26.6 ± 6.4 ($p < 0.05$). In simultaneous experiments with adult controls, interferon increased MC-NKC from 33.3 ± 12.6 to 53.0 ± 10.4 ($p < 0.05$).

To further define MC-NKC, the use of a second target cell, K562, was undertaken (Table III). NKC to K562 in a 4-hr Cr-release assay using cells from patients one, three, and four was lower than that of normal adult controls ($p < 0.05$ at all effector to target cell ratios). As noted against HSV-infected cells, patient four had the highest levels of NKC to K562 target cells among the patients tested.

Effect of Mab directed against leukocyte surface glycoproteins on cytotoxicity. To further define the role of LFA-1/OKM-1 in cellular cytotoxicity to HSV-infected target cells, leukocytes from normal human controls were incubated with $F(ab')_2$ fragments of several MAb or OKM-

TABLE III

NKC of patients with leukocyte LFA-1/OKM-1 deficiency to K562 target cells

Patient	E:T[a]	Patient	Control[b]
1	100	24.5	71.2
	60	18.9	62.2
	30	0.0	66.8
3	100	5.7	74.5
	60	4.5	64.2
	30	0.0	42.5
4	100	35.5	78.4
	60	27.5	64.4
	30	27.3	49.8

[a] Effector to target cell ratio in a 4-hr ^{51}Cr-release cytotoxicity assay.
[b] Controls were unrelated adults.

TABLE IV

Inhibition of cytotoxicity by Mab directed against cell surface components[a]

Monoclonal Antibodies Against[b]	% Inhibition of Cytotoxicity	
	PMN-ADCC[c]	MC-NKC[d]
LFA-1-α	66.6 ± 8.5[e]	21.0 ± 10.6
LFA-1-β	17.0 ± 7.9	20.8 ± 10.2
LFA-1-α + LFA-1-β	73.9 ± 8.0[e]	34.7 ± 10.9[e]
OKM-1	10 ± 10.0	0 ± 0
OKM-1 + LFA-1-β	45.8 ± 14.7[e]	42.8 ± 6.2[e]
CR1	9.8 ± 5.3	11.0 ± 7.0

[a] The data are presented as mean ± SEM of four to seven experiments showing percent inhibition by Mab:

$$\frac{\text{percent control cytotoxicity} - \text{percent cytotoxicity in presence of monoclonal}}{\text{percent control cytotoxicity}}$$

[b] Leukocytes were incubated in phosphate-buffered saline (control) or designated Mab $F(ab')_2$ fragments and OKM-1 IgG.
[c] PMN-ADCC, effector to target cell ratio of 100:1.
[d] Mononuclear leukocyte NKC, effector to target cell ratio of 100:1.
[e] Cytotoxicity values compared with control, $p < 0.01$ by using one-tailed Student's paired t-test.

1 IgG, were washed, and were tested in the cytotoxicity assay (Table IV). The results depicted in Table IV are from experiments utilizing an effector to target cell ratio of 100:1; similar findings were observed at ratios of 60:1 and 30:1.

MAb against LFA-1-α significantly inhibited PMN-ADCC. Neither anti-OKM-1 nor anti-LFA-1-β alone induced significant inhibition of cytotoxicity. Combinations of anti-LFA-1-α and anti-LFA-1-β or of anti-OKM-1 and anti-LFA-1-β induced marked and significant inhibitory effects on PMN ADCC and MC-NKC (Table IV). In addition, the combination of MAb to the α- and β-subunit of LFA-1 induced 62.1 ± 6.4% ($p < 0.05$) inhibition of MC-ADCC. A binding control antibody to the CR1 receptor of complement had no significant inhibitory effects on cytotoxicity. A similar lack of inhibition was seen by using an HLA-framework MAb. In the case of anti-LFA-1-β the use of whole antibody, as well as its $F(ab')_2$ fragment did not cause significant inhibition of cytotoxicity, unless combined with anti-OKM-1 or anti-LFA-1-α (data not shown). In several experiments, inclusion of MAb in the cytotoxicity assay, after preincubation of effector cells in the same MAb, was not associated with further or significant increase in the degree of inhibition of cytotoxicity.

DISCUSSION

We have described cytotoxicity studies involving leukocytes from four patients with a hereitable immunodeficiency syndrome involving recurrent pyogenic infections and leukocytosis. *In vitro,* these patients' PMN failed to mediate many adhesive-dependent functions, such as phagocytosis, chemotaxis, adherence, aggregation, and rosetting with C3bi-coated erythrocytes (17). These patient's leukocytes were shown to be markedly deficient in several high m.w. membrane glycoproteins that react with Mab against LFA-1 and OKM-1 (17).

The molecular deficiency in this disorder appears to be highly specific and limited to the LFA-1, OKM-1 glycoprotein family. Other surface molecules, including the complement receptor 1 (CR1), HLA antigen glycoproteins, and the IgG Fc receptor, are present in normal amounts on granulocytes and monocytes of these patients and also demonstrate normal mobility in SDS-PAGE (17, 26–28)[4] The integrity of the Fc receptor on granulocytes of these patients has been confirmed by using FACS analysis employing specific anti-Fc receptor MAb,[4] immunoprecipitation of ^{125}I-labeled surface proteins by this MAb,[4] and in addition, by using functional analyses in phagocytosis and spreading assays (17, 26). The kinetics of ingestion of particles or microorganisms specifically opsonized with IgG and the extent of spreading on immune complex-coated substrates by LFA-1/OKM-1-deficient granulocytes are normal, as compared with healthy adult granulocytes assayed under the same conditions; such findings suggest normal Fc-facilitated functional activity (17).

In vitro, the PMN of these patients were deficient in ADCC to HSV-infected cells over a broad range of effector ratios (Table I) and target cell antibody-sensitization ranges (Fig. 1). Interferon had no effect on patients' or controls' PMN ADCC (data not shown). By using a single cell conjugation assay it was demonstrated that the ADCC defect was due, at least in part, to an inability of the patients' PMN to adhere to target cells in the presence of antibody (Table III). Thus, even with normal immunoglobulin Fc receptor function (17), lack of these glycoproteins excluded normal PMN-ADCC. Because there was very little adherence of normal PMN to target cells in the absence of antibody (Table III), these studies have defined an adherence step mediated by LFA-1/OKM-1 that probably occurs simultaneously or after the PMN Fc receptor and immunoglobulin interaction, before the lysis step. It is also possible that Fc receptors and a component of the LFA-1/OKM-1 complex each adhere weakly to ligands on the target cells, and a combination of these activities are required to mediate cytotoxicity.

By using $F(ab')_2$ fragments of MAb or OKM-1 IgG directed against the α-chains and β-chains of the LFA-1 and OKM-1 leukocyte surface glycoproteins of normal PMN, we have been able to duplicate the PMN-ADCC defects of these patients (Table IV). It appears that anti-LFA-1-α alone, or a combination of anti-LFA-1-α and anti-LFA-1-β or anti-OKM-1 and LFA-1-β are quite effective in blocking PMN-ADCC. These data further confirm the roles of these glycoproteins in normal PMN-ADCC.

In vitro, the MC of patients one, two, and three were deficient in NKC to HSV-infected and K562 target cells. Patient four, who is older and whose deficiency of OKM-1 and LFA-1 antigens on the leukocyte surface is less deficient quantitatively (18), had the highest NKC activity against both substrates (Tables I and III). These data are concordant with the literature that has recorded defective NKC (29) and normal NKC (30) mediated by cells of LFA-1/OKM-1-deficient patients. More finite analysis regarding the quantity of surface glycoprotein in each patient will probably clarify this apparent contradiction.

Interferon increased NKC of patients and controls, as previously reported in normal humans and certain humans with low anti-HSV NKC, such as neonates (31). Interferon stimulates NKC by increasing lysis of already rosetted cells, but does not increase effector cell adhesion (6, 23). Thus, these patients' interferon-stimulated NKC mechanisms appear intact.

The basic cause of the NKC defect in patients one to three is difficult to discern, due to low numbers of conjugates formed in the single cell agarose assay using MC without antibody (Table II). The apparent lack of a difference between the number of conjugates seen in MC from patients and the number of conjugates seen in controls must be confirmed by tests of enriched NKC cells, before a firm judgement regarding an adhesive or lytic defect can be made. MAb against LFA-1-α and LFA-1-β chains or against OKM-1 and LFA-1-β significantly inhibited NKC of MC from normal humans (Table IV). Thus, we had simulated the defect seen in these patients with relevant MAb. Anti-LFA-1-α and anti-LFA-1-β, but not anti-OKM-1 monoclonals, have been previously reported to block NKC (14).

Unlike the PMN of patients with LFA-1/OKM-1 defect, MC from these patients mediated normal levels of ADCC (Table I). As expected, the single cell assay demonstrated that antiviral immunoglobulin increased the number of

[4] **Springer, T. A., W. S. Thompson, L. J. Miller, F. C. Schmalstieg, and D. C. Anderson.** 1984. Inherited deficiency of the LFA-1, Mac-1, P150,95 glycoprotein family: and its Molecular Basis. J. Exp. Med. (in press).

MC target cell conjugates (Table II). LFA-1 and OKM-1 are normally present on NKC cells and macrophages (20, 21), the two major effector cells of MC-ADCC to HSV-infected target cells (22, 32). A more detailed analysis of these specific cell populations in the LFA-1/OKM-1 patients will be necessary to explain abnormal MC-NKC and normal MC-ADCC. It is possible that in these patients, MC-ADCC, unlike NKC, is mediated primarily by a different cell (as a macrophage) that is less severly affected by the glycoprotein defect. Alternatively, it is possible that PMN-ADCC and MC-ADCC do not require the same surface type or quantity of adhesive glycoproteins. The demonstration that relevant MAb (a combination of anti-LFA-1-α and anti-LFA-1-β) had significant but low inhibitory effects on MC-ADCC suggests that the latter is possible. Several previous studies have demonstrated an enhancing role of complement or the C3bi receptor for MC-ADCC (33, 34). The C3bi receptor appears to be identical to the antigen detected by anti-OKM-1 (17, 21, 30).

The lymphocyte function associated-1 (LFA-1), Mac-1, and p150,95 molecules constitute a family of structurally and functionally related high m.w. human leukocyte surface glycoproteins. Each molecule contains an α-subunit and a β-subunit noncovalently associated in $\alpha_1\beta_1$-structure. They are distinguished by their α-subunits, which have different isoelectric points, m.w., and cell distributions, and are immunologically specific. The LFA-1, Mac-1, and p150,95 α-subunits include α-L of 177,000 M_r, α-M of 165,000 M_r, and α-X of 150,000 M_r, respectively. The β-subunits of M_r of 95,000, in each of these three molecules, are identical (21). Mac-1 was first identified by using MAb as a mouse differentiation-antigen present on myeloid cells but absent on lymphoid cells (35). Human Mac-1 was later identified by cross-reaction of a MAb with mouse Mac-1 (36). A number of MAb to other determinants on human Mac-1 were later prepared, including OKM-1, Mo-1, OKM-9, and OKM-10 (30, 37). Mac-1-α (equivalent to OKM-1) appears to be identical to the complement receptor type 3 (CR3). The CR3 binds the inactivated form (iC3b) of the third component of complement and mediates adherence and phagocytosis of C3bi-coated particles by granulocytes and monocytes (38). Some MAb to Mac-1 inhibit the CR3 on myeloid cell surfaces (37–39), and *Staphylococcus aureus*-MAb-Mac-1 complexes formed with noninhibitory MAb and soluble Mac-1 specifically agglutinate iC3b-opsonized erythrocytes (37).

The results of our studies further define the leukocyte defects in this immunodeficiency disease to include PMN-ADCC and variable MC-NKC defects. Whereas previous data generated by using MAb-blocking experiments have implied the importance of LFA-1 in cytotoxicity (9–14), these are in first *in vivo* human data linking defective cytotoxicity with a genetic deficiency of LFA-1/OKM-1. Further experience with this new clinical-pathologic model may demonstrate high risk for systemic viral infection and/or the development of tumors, thus supporting the antiviral and antitumor role of LFA-1/OKM-1-mediated cytotoxic mechanisms *in vivo*. Several adherence-mediating proteins have been shown to be important in T cell and NK cytotoxicity (9–14). This new defect has allowed us to substantiate the role of adherence-mediating proteins in ADCC and NKC of human PMN and MC.

Acknowledgments. We would like to acknowledge the technical assistance of Bonnie J. Hughes and the word processing expertise of Beverly Archuleta and Krysti Clemons.

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